The role of CD147 in liver injury: "The truth is in the details"

To the Editor

We have read the manuscript by Zhang *et al.* with great interest [1]. The results demonstrate an important role for CD147 in liver injury. However, we have published results, which are not referenced by Zhang *et al.* that appear to contradict some of their findings [2]. Our immunohistochemistry staining for CD147 shows a pattern of expression consistent with hepatocyte and leukocyte specific expression, but with negligible expression by hepatic stellate cells (HSC) [2]. We used a well-characterised commercial monoclonal antibody (MEM-M6/1) [2,3], whilst HAb18G, the antibody used in the manuscript, is not commercially available [1]. Confocal microscopy of CD147, using the antibody MEM-6/1, shows localisation with the CK-18 marker of hepatocytes, but not with α-smooth muscle actin (α-SMA) expressed by activated HSC (Fig. 1). The staining presented in Fig. 1A of the manuscript by Zhang *et al.* shows dense fibrous bands in cirrhosis that are strongly α-SMA positive, as expected [1]. However, the adjacent panels in Fig. 1A of cirrhosis, stained with HAb18G for CD147, have a different pattern of expression [1]. Therefore, we question the association of CD147 and α-SMA expression in the liver. A possible explanation is the heterogeneity in HSC populations as discussed by Zhang *et al.* [1]. However, we have not observed such heterogeneity; there is no significant confocal microscopy co-localisation of MEM-6/1-stained CD147 expressing cells and α-SMA positive cells (Fig. 1). Further, we observe staining of hepatocytes [2], which is not demonstrated in this manuscript (Fig. 1). We have confirmed these results by showing significant *CD147* mRNA expression in primary isolated hepatocytes, which is comparable to whole liver tissue expression. Our results are in agreement with those published on the Human Protein Atlas [4], and are consistent with data produced by other groups, showing CD147 expression in hepatocyte-derived cell lines [5,6]. A plausible explanation is, given that there are four major CD147 isoforms combined with varying glycosylation modifications of this widely expressed protein, that the different forms of CD147 are selectively targeted by different antibodies. Therefore, we believe the pattern of CD147 expression presented in the manuscript should be interpreted as being specific for the antibody used, and not generalised for all CD147 expression. It would be useful to have both high and low power views of the direct immunofluorescent staining of α-SMA and CD147 co-localisation. Additionally, Zhang *et al.* show in panel 3D that in the absence of CD147 overexpression, the HSC line LX-2 does not express α-SMA [1]. However, in the original manuscript, in which LX-2 where isolated [7], and subsequent articles, it has been shown that LX-2 cells have an activated phenotype and do express α-SMA [8]. Further, Zhang and colleagues have demonstrated that activated LX-2 HSC *in vivo* lack significant CD147 protein expression, when assessed by Western blot analysis (Fig. 3D) [1]. Importantly, LX-2 exhibit multipotent behaviour and characteristics of bone marrow-derived mesenchymal stem cells and may not be the best model of *in vivo* HSC activation [9]. We commend the authors for this important work but think the role of CD147 in liver fibrosis is considerably more complex than that presented by Zhang *et al.* [1].

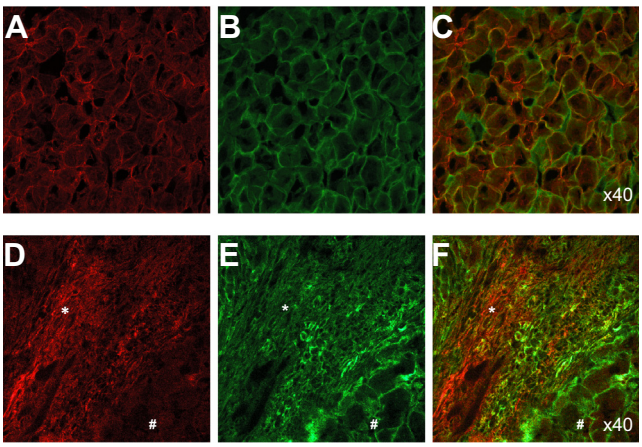

Fig. 1. Confocal microscopy of CD147 with hepatocyte and hepatic stellate cell (HSC) markers. Confocal microscopy was performed on cirrhotic tissue with hepatocytes identified by CK-18 staining (red, in A) and HSC by α-SMA (red, in D). CD147 was stained with the monoclonal antibody MEM-6/1 (green in B and E). (C) The merged image of hepatocyte CK-18 and CD147 staining demonstrates co-localisation. In panels D-F there is clear staining of HSC in the fibrotic septa by α-SMA (example marked with ∗) and staining of CD147 (example marked by #). (F) In the merge image of hepatic stellate cell and CD147 staining, there is no significant co-localisation.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Reply to: "The role of CD147 in liver injury: The truth is in the details"

To the Editor:

We greatly appreciate the letter by McLennan *et al*., which discusses our recently published article on the role of HAb18G/CD147 promoting liver fibrosis through activation of hepatic stellate cells (HSCs) [1].

In a chronically injured liver, caused by hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol or other factors, HSCs are activated by inflammation mediators and differentiate into myofibroblasts, which play an important role in the development of liver fibrosis and cirrhosis. In China, HBV causes more cases of liver cirrhosis than HCV, therefore our research interest focused on HBV-related liver cirrhosis in our article [1]. The published article by Shackel *et al.* using array analysis compared gene expression of HCV-associated cirrhosis with autoimmune hepatitis-associated cirrhosis, showing a marked difference in the apoptosis-associated gene profile, reflecting the fact that different pathogens lead to different gene expression profiles.

However no data were shown in HBV-associated cirrhosis [2].

The antibody HAb18, for immunohistochemistry (IHC) of human liver cirrhosis tissue, mentioned in our article, has already been used for years in our laboratory, is qualified for Western blot, IHC, immunofluorescent staining, and has been granted international patents (Patent Numbers: PCT/CN03/00188 and PCT/CN2002/000356) [3,4]. The fragment $F(ab')_2$ of HAb18 labeled with ^{131}I, registered as Licartin, has been approved for the treatment of hepatocellular carcinoma (HCC) by the China State Food and Drug Administration (Registration No. S20050039) [5]. HAb18 combines with the Ig-C2 domain of isoform *Basigin-2,* which is the most predominant splice variant, encoding the well-known EMMPRIN/CD147, but not with isoform *Basigin-3* [6,7].

Our previous IHC with commercial mouse anti-human CD147 antibody (NovoCastra) showed immunostaining in the tumor compartment, but not in the stromal compartment, in HCC tissues, which is consistent with our HAb18 staining in cirrhotic tissues, where HAb18G/CD147 was absent in the fibrous septa area [8,1]. In the mouse liver fibrosis model, CD147 expression in activated HSCs was shown with a commercial rat anti-mouse CD147 antibody (Abcam ab34016) by co-localization with alpha-smooth muscle actin (α-SMA) in Disse's space [1]. In our previous study we showed that HAb18G/CD147 was expressed in hepatocytes of cirrhotic and HCC tissues, consistent with Shackel's paper [9,2]. The α-SMA-positive cells represent a heterogeneous population, including activated resident fibroblasts, activated HSCs, bone marrow-derived mesenchymal cells and fibrocytes, hepatocytes or biliary epithelium-derived fibroblasts by epithelial-mesenchymal transition, which means that not all of the α-SMA-positive cells are derived from HSCs. Therefore, we identified HSCs by reaction with α-SMA and their localization in the perisinusoidal space. McLennan *et al.* showed a positive staining of α-SMA by immunofluorescence in fibrotic septa while no immunoreaction with α-SMA was observed in the perisinusoidal space (Fig. 1D). Importantly, fibrous septa composed of collagen have a strong autofluorescence, the specific fluorescence is suggested to be identified by blank and isotype controls. In Fig. 1C of the McLennan's study, the co-localization of CD147 with CK-18 was observed in hepatocytes, but no CK-18 expression was observed in some CD147 positive cells detected by green fluorescence.

LX-2 cell line was originally isolated with property of α-SMA expression detected by immunofluorescence [10]. We also observed a weak immunofluorescent signal of α-SMA expression in our work. However, we did not observe a detectable level of α-SMA protein expression by Western blot, but mRNA expression was detected by real-time PCR in untreated LX-2 cells. Stable transfection with the *CD147* gene in LX-2 cells induced a notable α-SMA expression [1]. This may be due to a different detection sensitivity and different sources of primary and secondary antibodies.

In summary, we have reasons to believe that the HAb18 antibody used by us works properly and that the conclusions drawn from these experiments are valid.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.